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Feeding history effects on feeding responses of *Rhagoletis indifferens* (Dipt., Tephritidae) to GF-120 and Nulure

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Abstract: Effects of feeding history on feeding responses of western cherry fruit fly, *Rhagoletis indifferens* Curran, to the commercial protein baits GF-120 and Nulure were determined in the laboratory. Flies were kept on 5% sucrose alone or yeast extract and sucrose (Y + S) for 3–7 or 14–16 days and exposed to 24-h-old GF-120 or Nulure drops on artificial leaves. Numbers and durations of feeding events on leaves and durations of non-feeding events were recorded over 1-h periods. Experiments were also conducted to determine effects of Y + S feeding sequences on responses to Nulure, of starvation after sucrose or Y + S feeding on responses to Nulure, and of feeding history on mortality after exposure to GF-120 and Nulure. Protein-deprived flies consistently fed more times on GF-120 and Nulure than protein-fed flies and fed longer. One day of exposure to Y + S or 16 h of starvation after exposure to sucrose caused greater feeding on Nulure than 7 days of exposure to Y + S or 16 h of starvation after exposure to Y + S. Durations of non-feeding events on leaves with sucrose or bait were similar in protein-deprived and -fed flies. Responses of 4- to 6-day-old flies kept on sucrose to 0- and 24-h-old GF-120 or Nulure were similar. More flies kept on sucrose were paralysed or dead at 6–32 h after exposure to GF-120 or Nulure with spinosad than flies kept on Y + S. Results show that complete or long periods of protein deprivation and starvation after sucrose feeding increased feeding responses to GF-120 and Nulure. The general lack of differences in durations of non-feeding events on leaves with sucrose or GF-120 or Nulure in protein-deprived and -fed flies suggests that most protein-deprived flies found baits through chance encounters following normal movement.

Keywords: protein baits, sucrose, western cherry fruit fly, yeast extract

1 Introduction

The western cherry fruit fly, *Rhagoletis indifferens* Curran, a major insect pest of sweet cherries, *Prunus avium* (L.) L., is currently managed in many areas in Washington State using sprays of GF-120 Fruit Fly Bait (Dow AgroSciences, Indianapolis, IN). This bait, which contains a mixture of protein, sugar, and the toxin spinosad, has, in many cases, replaced organophosphate and carbamate insecticides for control of this fly. Bait or insecticide sprays continue to be necessary because *R. indifferens* is a quarantine pest and there is a zero tolerance for its larvae in commercial fruit. Most commercial cherry orchards probably have few or no flies, but sprays are applied as insurance and because feral or abandoned cherry trees near orchards can be sources of infestations.

While GF-120 appears effective, it generally does not eliminate larval infestations by *R. indifferens* (Yee and Chapman 2005) and other *Rhagoletis* species (Van Steenwyk et al. 2003; Pelz et al. 2005). The reasons for this are unclear, but the variable and generally low responses of tephritid flies to protein baits (Prokopy et al. 1993; Vargas et al. 2002; Barry and Polavarapu 2004; Revis et al. 2004; Pelz et al. 2005) may be one reason. If flies are attracted more quickly to baits, control using sprays might be more effective because they would kill more flies before they oviposit into fruit. Nulure (Scentry Biologicals, Billings, MT) is a protein-based bait that has been used for many years for tephritid fly management and like GF-120 elicits variable responses. Some *R. indifferens* flies readily feed on bait drops placed 1–2 cm from them, whereas others do not feed: the percentages of flies that responded to GF-120 and Nulure within 5 min were 49.0% ($n = 47$) and 39.0% ($n = 41$), respectively (W. L. Yee, unpublished data). Given these similar responses and that Nulure is roughly one-fourth the cost of GF-120, an investigation into the responses of *R. indifferens* to Nulure should be conducted.

Little work has focused on the factors determining the variable feeding responses of *R. indifferens* to protein baits. It is likely that the feeding history of this species is one factor. In the laboratory, *R. indifferens* flies starved for 16–20 h were more attracted to

leaves with GF-120 drops than non-starved flies, although only 8% of starved flies responded. Protein deprivation alone in the presence of sucrose was insufficient to increase attraction (Yee and Chapman 2005). However, in at least four other tephritids, protein feeding reduces feeding responses to protein baits {(Mediterranean fruit fly, *Ceratitis capitata* [Wiedemann] (Prokopy et al. 1992; Vargas et al. 2002; Barry et al. 2003); apple maggot, *Rhagoletis pomonella* [Walsh] (Hendrichs et al. 1990a; Prokopy et al. 1993, 1994); Mexican fruit fly, *Anastrepha ludens* [Loew] (Robacker 1991); Queensland fruit fly, *Bactrocera tryoni* [Frogatt] (Prokopy et al. 1991); and oriental fruit fly, *Bactrocera dorsalis* [Hendel] (Vargas and Prokopy 2006)}. Determining the importance of various feeding histories on responses by female and male *R. indifferens* to various commercial protein baits will improve our understanding of the mechanisms by which protein baits function. It cannot be assumed that responses of temperate fruit flies such as *R. indifferens* will be identical to those of subtropical fruit flies mentioned above.

In this study, effects of various feeding histories on close-range attraction and feeding responses of *R. indifferens* to GF-120 and Nulure were determined in the laboratory. I tested the hypotheses that: (1) Flies deprived of protein are more responsive to GF-120 and Nulure than to sucrose, and are more responsive than flies not deprived of protein (response = feeding on baits); (2) females are more responsive to GF-120 and Nulure than males; (3) flies deprived of protein for long periods respond more to Nulure than flies deprived for shorter periods; and (4) flies starved after continuous sugar feeding respond more to Nulure than flies starved after continuous protein and sugar feeding.

2 Materials and Methods

Infested sweet and sour cherries were collected in June and July 2004 and 2005 from residential trees in central Washington State. Cherries were laid on hardware cloth suspended in tubs containing soil (equal volumes of sand, peat moss and vermiculite). Larvae dropped from cherries into the soil and pupated. Puparia were chilled at 3°C for 4–8 months. They were then transferred to 25–27°C, 30–40% RH, and under a 16 h light : 8 h dark cycle for adult emergence. Adults were maintained at these conditions before and during all tests except for one in experiment 7 (see below).

In experiment 1, undiluted blank GF-120 (without spinosad) was mixed with water to make a 40% (v : v) concentration (recommended 1 : 1.5 dilution). In experiments 2–4, Nulure (without insecticide) was tested at 100% concentration. Bait without insecticide was used because it was easier to handle and to dispose of than bait with insecticide, and there is no evidence that spinosad affects feeding responses. In the blueberry maggot, *Rhagoletis mendax* Curran, there were no feeding deterrent effects of SolBait (bait similar to GF-120) containing spinosad compared with control SolBait without insecticide, as mean feeding durations on them were almost the same (about 80 s during a 5-min bioassay) (Barry and Polavarapu 2005) (also see experiment 5 below to support this finding with *R. indifferens*). In all four experiments, six 25–33 μl drops of bait or 13% sucrose (= amount in 40% GF-120) were applied to an artificial dark green silk leaf (11.2 cm long × 9.1 cm wide, 76.1 cm^2) (Silk Gardens Shop, Irving, TX). Three drops of bait or sucrose were placed equidistantly on each half of the leaf. Drops were allowed to dry for 24 h at 19–21°C and 30–40% RH in a fume hood before being tested. Preliminary 1-h tests suggested that fewer flies landed on artificial leaves placed on the bottoms than on the sides of test containers. Attaching leaves to the sides of cages required dried bait to prevent run-off. It was also easier to see the mouthparts of a fly contacting the drop when the fly was viewed from the side than from above. Experiment 5 compared fly responses to 24-h-old baits with and without spinosad. Experiment 6 was conducted comparing fly responses to fresh and 24-h-old baits. Experiment 7 compared effects of different feeding histories on numbers of flies paralysed or dead after various hours post-exposure to 24-h-old baits with spinosad. All seven experiments were conducted 4–10 h after the beginning of photophase.

2.1 Feeding history, sex and responses to GF-120

In experiment 1, newly-emerged flies were placed into 473-ml paper cartons (7 cm high × 5 cm diameter) (Neptune Paper Products, Newark, NJ). Flies were kept on either a (1) 5% sucrose solution (w : w) on cotton wicks (no protein) or a (2) dried yeast extract (EZ Mix, Sigma, St Louis, MO) + sucrose diet (1 : 4 mixture, w : w) on paper strips (protein). Yeast extract was mixed with sucrose to ensure high intake of protein, as initial observations suggested yeast extract alone stimulated little or no feeding. Water in cotton wicks was provided throughout. Ten to forty flies (about equal numbers of each sex) were held inside each carton. Flies in the two feeding histories were aged 3–7 days or 14–16 days and tested the following day (thus ages at testing were 4–8 and 15–17 days respectively). Each of the six drops of sucrose or GF-120 covered ~28 or ~26 mm^2 of leaf surface respectively. Age groups were not tested during the same time periods, so no statistical comparisons of fly age effects were made. By testing the flies at different periods, another variable, the chilling periods of puparia of younger and older flies, also differed. The older flies were tested later and were kept as puparia in the cold by up to 2 months longer than were puparia of younger flies (6 months vs. 8 months). Thus the two age groups of flies were treated differently before they were aged as adults.

Four feeding history, sucrose or GF-120 treatments were compared in experiment 1: (1) sucrose alone, exposed to sucrose (control bait); (2) sucrose alone, exposed to GF-120; (3) yeast extract + sucrose (Y + S), exposed to sucrose; and (4) Y + S, exposed to GF-120.

For testing, six flies (three of each sex) were introduced into a 1.9-l white paper container (11 cm high × 16.2 cm diameter) (Sweetheart Cup, Owings Mills, MD) containing a leaf with sucrose or GF-120 drops. The leaf was held on the side of the cage with a paper clip. The container was covered with a light bridal cloth with 1 mm^2 openings that allowed easy monitoring of the flies inside. Flies were introduced through a hole in containers using 5.0 × 1.4 cm glass vials. Flies rarely contacted the leaves as they were being introduced into containers, but if they did, they immediately flew off them. Flies were allowed to settle for 1 min. Continuous observations of the flies were then made for 60 min. No water was provided during this time. Containers were placed ~20 cm under 40 W fluorescent lights (CNR Robinson Lighting and Supply Co., Baltimore, MD) at a mean light intensity of ~4800 lumen/m^2. Responses recorded for each sex were: (1) numbers of feeding events on a leaf, (2)

durations of feeding events, and (3) durations of non-feeding events on leaves. A feeding event was either continuous contact of the proboscis with GF-120 or sucrose or intermittent contact in which the fly lifted its proboscis for < 5 s from the GF-120 or sucrose while remaining stationary. Brief contacts of $\sim$1 s of the proboscis with GF-120 or sucrose were not considered feeding events. A flashlight was used to illuminate flies when it was necessary to confirm proboscis contact with sucrose or GF-120 (i.e. to confirm a feeding event). Data on numbers of non-feeding visits were not presented because of the nature of the data collection method. During one visit on a leaf a fly sometimes fed several times, separated by non-feeding events; these data and those for flies that visited leaves but did not feed were pooled on data sheets. Durations of non-feeding events were recorded to determine whether flies stayed on GF-120-treated leaves longer than on sucrose-baited leaves, which would be evidence for arrestment. There were 10 replicates of all treatments.

2.2 Feeding history, sex and responses to Nulure

The procedures in experiment 2 were identical to those in experiment 1, except that Nulure was tested rather than GF-120. Each of the six drops of Nulure covered $\sim$25 mm^2 of leaf surface. Four feeding history, sucrose, or Nulure treatments were compared: (1) sucrose alone, exposed to sucrose; (2) sucrose alone, exposed to Nulure; (3) Y + S, exposed to sucrose; and (4) Y + S, exposed to Nulure. There were 10 replicates of all treatments.

2.3 Feeding sequences and responses to Nulure

In experiment 3, general procedures were identical to those in experiments 1 and 2. However, 10 flies (five of each sex) instead of 10–40 were maintained together prior to testing (of which three of each sex were used per replicate), fly age at testing was 8 days, and Y + S and sucrose feeding sequences were altered to see how this affected responses. Feeding sequence treatments were: (1) 7 days on Y + S; (2) 3 days on 5% sucrose, 1 day on Y + S, and 3 days on 5% sucrose; (3) 7 days on 5% sucrose; and (4) 1 day on Y + S, 6 days on 5% sucrose. In (2) and (4), 5% sucrose was removed when Y + S was present. All treatments included one water wick throughout the pre-test period. There were 11–13 replicates of each treatment.

2.4 Starvation and responses to Nulure

Procedures in experiment 4 were identical to those in experiment 3, except that feeding histories differed, as the objective was to determine how starvation after exposure to Y + S or sucrose alone affected responses to Nulure. Treatments were: (1) 6 days on 5% sucrose, 1 day on Y + S; (2) 6 days on Y + S, 1 day on 5% sucrose; (3) 6 days on 5% sucrose, 16 h starvation; and (4) 6 days on Y + S, 16 h starvation. There were 11 replicates of each treatment.

2.5 Responses to baits without and with spinosad

In *R. mendax*, there was no feeding deterrent effect of spinosad (Barry and Polavarapu 2005). However, to ensure that there were no effects of spinosad on feeding by *R. indifferens*, experiment 5 was conducted comparing responses of 5- to 7-day-old flies kept on 5% sucrose alone to blank GF-120 and to GF-120 with spinosad and comparing responses to Nulure and to Nulure with spinosad. An equivalent of 0.02% spinosad (w : v) (Entrust [80% spinosad]; Dow AgroSciences, Indianapolis, IN) was mixed with blank GF-120, the amount found in marketed GF-120. Spinosad was mixed and dissolved in water and then added to GF-120 to make a 40% GF-120 concentration. In a separate test, the equivalent of 0.02% spinosad was added to Nulure. Spinosad was mixed and dissolved in water and then added to make an 86% Nulure concentration. All treatments were aged for 24 h. Other methods followed those of previous experiments. In addition, numbers of paralysed or dead flies (pooled) were also recorded 20 h after testing to determine the numbers of flies that may have fed on baits with spinosad. Paralysed flies could move their legs but not walk straight when probed. Paralysed flies that were right side up (1) held their heads down or had heads twisted to the side, (2) held their wings at a 45° angle, and (3) were sprawled. Paralysed flies that were on their backs moved only their legs; dead flies showed no movement. Only water was provided for flies after testing with GF-120. Because Nulure had no added sugar and control mortality needed to be low, a 5% sucrose solution on a wick was provided for flies after testing with Nulure. There were 10 replicates for GF-120 and Nulure tests.

2.6 Responses to fresh vs. aged GF-120 and Nulure

Experiment 6 compared the responses of 4- to 6-day-old flies kept on 5% sucrose alone to 0- and 24-h-old GF-120 and to 0- and 24-h-old Nulure on artificial leaves. Tests of GF-120 and Nulure were conducted separately and not compared. Procedures were similar to those in all previous experiments, except for the following. An artificial leaf was glued (All Purpose Stik glue sticks; FPC Corp., Wauconda, IL) horizontally onto the top end of an upright 5.5 cm tall $\times$ 2.7 cm wide clear plastic vial that itself was glued onto the bottom of a container in the centre. Fresh (0 h) drops of bait were applied onto the top of the leaf $\leq$5 min before exposure to flies. A leaf was 5 cm below the top of the container, close enough to observe contact of the proboscis on baits from the side. Only flies on the top of the leaves were recorded. There were eight replicates for both GF-120 and Nulure tests.

A test was also conducted to determine whether feeding duration by 7- to 10-day-old flies kept on 5% sucrose alone on fresh GF-120 or Nulure with and without spinosad differed. Spinosad was added to GF-120 as described for experiment 5. Spinosad was added directly to Nulure without addition of water, but the spinosad to Nulure ratio was the same as in experiment 5. Unlike in experiment 5, both baits were tested fresh. A single fly was placed inside a 5 $\times$ 14 cm glass vial and allowed to settle for about 1 min. One 2 μl drop of bait was introduced. The fly was watched for 5 min and numbers of feeding events and feeding durations were recorded. The test was conducted at 21°C. Ten female and 10 male flies were tested for both GF-120 and Nulure treatments.

2.7 Feeding history and mortality of flies exposed to GF-120 and Nulure

Experiment 7 determined the mortality of 5- to 8-day-old flies kept on 5% sucrose alone or on Y + S and then exposed to GF-120 with spinosad and Nulure with spinosad. This experiment was conducted to provide evidence that more flies kept on sucrose feed on GF-120 or Nulure than flies kept on Y + S. The feeding response protocol used in experiments

1–6 could not determine the numbers of flies that fed (unless there was only one feed) because it was not possible to know if the same fly fed on drops if it left the leaf and more feedings occurred later. Spinosad was added to GF-120 and Nulure as described for experiment 6. Other materials and methods for the cage set-up and observations of responses were similar as in all previous experiments. Six bait drops were applied on each leaf and allowed to dry for 24 h before exposure to flies. Three male and three female flies were placed into each cage. One hour after introduction, one cotton wick with 5% sucrose was provided for flies in each cage. Numbers of paralysed or dead flies (= mortality, pooled) were counted at 2, 4, 6, 8, 11, 24 and 32 h after exposure. There were 10 replicates of each treatment. For consistency with previous experiments, GF-120 and Nulure data were analysed separately.

2.8 Statistics

Numbers of feeding events, durations of feeding events and durations of non-feeding events were analysed in experiments 1–6. For experiments 1 and 2, data were analysed using three-way analysis of variance (ANOVA), with fixed effects being feeding history (sucrose vs. Y + S), bait (sucrose vs. GF-120 or Nulure) and sex. Analyses for main effects were conducted, and when there were significant interactions, simple effects were tested with one-way ANOVA (Schabenberger 1998). Data in experiments 3 and 4 were analysed with one-way ANOVA (within sex) and two-way ANOVA, the latter using feeding history and sex as fixed effects. One-way ANOVA was followed by pairwise comparisons using Fisher's least significant difference test. Data in experiments 5 and 6 were analysed with two-way ANOVA, using bait with or without spinosad and sex and fresh or aged bait and sex as fixed factors respectively. Numbers of paralysed and dead flies at 20 h after exposure in experiment 5 and at 2, 4, 6, 8, 24 and 32 h after exposure in experiment 7 were analysed with one-way ANOVA. Numbers +1 were subjected to square-root transformation before analyses. The Statistical Analysis System (SAS Institute 2001) was used for analyses. Mean ± SE are reported.

3 Results

3.1 Feeding history, sex and responses to GF-120

Three-way ANOVA of data from 4- to 8-day-old flies (table 1) indicated feeding history and bait (GF-120 or sucrose) affected numbers of feeding events and durations of feeding events, but not durations of non-feeding events. Sex affected numbers of feeding events and durations of non-feeding events, as females fed more often and stayed longer on leaves than males. However, four feeding history × bait and feeding history × sex interactions indicated effects of feeding history differed depending on whether sucrose or GF-120 was the bait and depending on the sex of the flies. In 15- to 17-day-old flies (table 1), feeding history significantly affected all three response variables, bait affected only durations of non-feeding events, and sex had no effect. Unlike in 4- to 8-day-old flies, there were no significant interactions among factors.

Analyses of simple effects arising from the four significant interactions for 4- to 8-day-old flies (table 2) showed that flies kept on sucrose fed more on GF-120 than sucrose, feeding events on sucrose were greater in flies kept on sucrose than Y + S, and that females did not respond significantly more than males ($P = 0.0598$). Durations of feeding and non-feeding events were also affected similarly by feeding history (table 2).

3.2 Feeding history, sex and responses to Nulure

Three-way ANOVA of data from 4- to 8-day-old flies (table 3) indicated that feeding history affected numbers and durations of feeding events. Bait (Nulure or sucrose) affected all three response variables, and sex affected numbers and durations of feeds, as females fed more often and longer than males. However, there

Table 1. *Results of three-way analysis of variance testing effects of feeding history, bait and sex on responses to GF-120 by* Rhagoletis indifferens

	No. feeding events		Durations, feeding		Durations, non-feeding	
Factor	*F*	P	*F*	P	*F*	P
4- to 8-day-old flies						
Feeding history	45.9	< 0.0001	40.1	< 0.0001	0.8	0.3640
Bait*	7.7†	0.0070	4.6†	0.0349	0.4	0.5053
Sex	4.4†	0.0404	1.5†	0.2182	9.5	0.0029
Feeding history × bait*	7.7†	0.0070	4.6†	0.0349	6.3	0.0140
Bait* × sex	0.3	0.6127	0.2	0.6409	0.7	0.4146
Feeding history × sex	4.4†	0.0404	1.5†	0.2182	0.3	0.5885
Feeding history × bait* × sex	0.3	0.6127	0.2	0.6409	1.4	0.2430
15- to 17-day-old flies						
Feeding history	16.4	0.0001	14.1	0.0003	15.9	0.0002
Bait*	1.4†	0.2347	1.1†	0.2989	6.2	0.0149
Sex	1.3†	0.2501	0.4†	0.5404	0.5	0.5002
Feeding history × bait*	1.4†	0.2347	1.1†	0.2989	1.1	0.3026
Bait* × sex	0.0	0.9845	0.0	0.9748	0.1	0.8109
Feeding history × sex	1.3†	0.2501	0.4†	0.5404	0.1	0.7377
Feeding history × bait* × sex	0.0	0.9845	0.0	0.9748	0.04	0.8499

d.f. = 1, 73.
*GF-120 or sucrose.
†Repeated values because of lack of feeds in Y + S treatment.

Table 2. *Tests of simple effects arising from interactions in GF-120 experiment in table 1 in 4- to 8-day-old flies*

	F	P	Responses greater
No. feeding events			
Feeding history × bait			
Sucrose history: GF-120 vs. sucrose	7.2	0.0107	GF-120
Y + S history: GF-120 vs. sucrose	No feeding events	–	–
Sucrose bait: Y + S vs. sucrose history	13.9	0.0006	Sucrose history
GF-120 bait: Y + S vs. sucrose history	29.3	< 0.0001	Sucrose history
Feeding history × sex			
Sucrose history: females vs. males	3.8	0.0598	ns
Y + S history: females vs. males	No feeding events	–	–
Males: Y + S vs. sucrose history	11.4	0.0017	Sucrose history
Females: Y + S vs. sucrose history	29.2	< 0.0001	Sucrose history
Durations of feeding events			
Feeding history × bait			
Sucrose history: GF-120 vs. sucrose	4.7	0.0368	GF-120
Y + S history: GF-120 vs. sucrose	No feeding events	–	–
Sucrose bait: Y + S vs. sucrose history	9.8	0.0034	Sucrose history
GF-120 bait: Y + S vs. sucrose history	30.5	< 0.0001	Sucrose history
Durations of non-feeding events			
Feeding history × bait			
Sucrose history: GF-120 vs. sucrose	1.1	0.3082	ns
Y + S history: GF-120 vs. sucrose	No feeding events	–	–
Sucrose bait: Y + S vs. sucrose history	4.0	0.0517	Sucrose history
GF-120 bait: Y + S vs. sucrose history	1.7	0.1974	ns

d.f. = 1, 38; ns, not significant.

Table 3. *Results of three-way analysis of variance testing effects of feeding history, bait and sex on responses to Nulure by* Rhagoletis indifferens

	No. feeding events		Durations, feeding		Durations, non-feeding	
Factor	*F*	P	*F*	P	*F*	P
4- to 8-day-old flies						
Feeding history	22.0	< 0.0001	28.2	< 0.0001	3.0	0.0871
Bait*	44.2	< 0.0001	44.7	< 0.0001	30.8	< 0.0001
Sex	4.7	0.0327	7.2	0.0089	0.4	0.5348
Feeding history × bait*	17.4	< 0.0001	19.0	< 0.0001	0.02	0.8957
Bait* × sex	2.8	0.1007	3.1	0.0845	0.6	0.4456
Feeding history × sex	4.3	0.0414	5.1	0.0276	0.3	0.5762
Feeding history × bait* × sex	2.4	0.1226	1.7	0.1950	0.2	0.6463
15- to 17-day-old flies						
Feeding history	25.0	< 0.0001	25.4	< 0.0001	1.3	0.2604
Bait*	32.1	< 0.0001	33.8	< 0.0001	0.5	0.4717
Sex	1.8	0.1801	1.2	0.2871	0.8	0.3722
Feeding history × bait*	25.0	< 0.0001	25.4	< 0.0001	0.4	0.5520
Bait* × sex	1.8	0.1801	1.2	0.2871	0.5	0.4992
Feeding history × sex	4.1†	0.0477	3.4†	0.0688	0.4	0.5295
Feeding history × bait* × sex	4.1†	0.0477	3.4†	0.0688	2.0	0.1574

d.f. = 1, 73.
*Nulure or sucrose.
†Repeated values because of lack of feeds in Y + S treatment.

were four significant feeding history × bait and feeding history × sex interactions for numbers and durations of feeding events. In 15- to 17-day-old flies, feeding history and bait each affected numbers and durations of feeding events (table 3). Sex had no effect. There were three significant feeding history × bait interactions for numbers and durations of feeding events. In general, analyses of simple effects arising from interactions in 4- to 8- and 15- to 17-day-old fly responses (table 4) showed that flies kept on sucrose responded more to Nulure than sucrose and female flies were not significantly more responsive than male flies.

3.3 Feeding sequences and responses to Nulure

Females kept for 7 days on sucrose or 1 day on Y + S followed by 6 days on sucrose fed 12.0 times more often than females kept for 7 days on Y + S ($F = 4.9$; d.f. = 3, 43; P = 0.0050) (fig. 1a). While the number of feeding events on Nulure by females kept for 3 days on sucrose, 1 day on Y + S and 3 days on sucrose was not statistically different from other treatments, numerically it was 5.7 times higher than that of flies kept for 7 days on Y + S and 2.1 times lower than of flies kept for 7 days on sucrose and flies kept for 1 day

Table 4. *Tests of simple effects arising from interactions in Nulure experiment in table 3*

	F	P	Responses greater
No. feeding events: 4- to 8-day-old			
Feeding history × bait			
Sucrose history: Nulure vs. sucrose	31.2	< 0.0001	Nulure
Y + S history: Nulure vs. sucrose	7.8	0.0083	Nulure
Sucrose bait: Y + S vs. sucrose history	2.1	0.1544	ns
Nulure bait: Y + S vs. sucrose history	17.8	0.0001	Sucrose
Feeding history × sex			
Sucrose history: females vs. males	2.8	0.0996	ns
Y + S history: females vs. males	0.01	0.9185	ns
Males: Y + S vs. sucrose history	3.0	0.0938	ns
Females: Y + S vs. sucrose history	9.2	0.0044	Sucrose history
Durations of feeding events: 4- to 8-day-old			
Feeding history × bait			
Sucrose history: Nulure vs. sucrose	39.6	< 0.0001	Nulure
Y + S history: Nulure vs. sucrose	8.4	0.0063	Nulure
Sucrose bait: Y + S vs. sucrose history	2.0	0.1619	ns
Nulure bait: Y + S vs. sucrose history	26.0	< 0.0001	Sucrose
Feeding history × sex			
Sucrose history: females vs. males	3.3	0.0793	ns
Y + S history: females vs. males	0.4	0.5587	ns
Males: Y + S vs. sucrose history	3.8	0.0591	ns
Females: Y + S vs. sucrose history	11.7	0.0015	Sucrose
No. feeding events: 15- to 17-day old			
Feeding history × bait			
Sucrose history: Nulure vs. sucrose	27.1	< 0.0001	Nulure
Y + S history: Nulure vs. sucrose	2.1	0.1544	ns
Sucrose bait: Y + S vs. sucrose history	No feeding events	–	–
Nulure bait: Y + S vs. sucrose history	22.7	< 0.0001	Sucrose history
Feeding history × sex			
Sucrose history: Females vs. males	1.6	0.2075	ns
Y + S history: Females vs. males	2.1	0.1544	ns
Males: Y + S vs. sucrose history	3.6	0.0645	ns
Females: Y + S vs. sucrose history	10.6	0.0024	Sucrose history
Durations of feeding events: 15- to 17-day old			
Feeding history × bait			
Sucrose history: Nulure vs. sucrose	35.7	< 0.0001	Nulure
Y + S history: Nulure vs. sucrose	1.7	0.1985	ns
Sucrose bait: Y + S vs. sucrose history	No feeding events	–	–
Nulure bait: Y + S vs. sucrose history	26.9	< 0.0001	Sucrose history

d.f. = 1, 38; ns, not significant.

on Y + S followed by 6 days on sucrose. In males, a similar overall pattern was seen. Males kept 1 day on Y + S followed by 6 days on sucrose fed 10.0 times more than flies kept for 7 days on Y + S ($F = 2.8$; d.f. = 3, 43; P = 0.0544) (fig. 1b). Females and males kept for 7 days on sucrose fed longer than flies kept for 7 days on Y + S (females: $F = 4.1$; d.f. = 3, 43; P = 0.0121; males: $F = 3.2$; d.f. = 3, 43; P = 0.0317) (fig. 1c,d). There were no differences in durations of non-feeding events by females or males among treatments (P > 0.05) (fig. 1e,f).

When analysed with two-way ANOVA, feeding sequence had effects on numbers of feeding events ($F = 7.4$; d.f. = 3, 86; P = 0.0002) and durations of feeding events ($F = 7.3$; d.f. = 3, 86; P = 0.0002), but not on durations of non-feeding events (P > 0.05). There was no sex effect and no treatment × sex interaction (P > 0.05).

3.4 Starvation and responses to Nulure

Females and males kept on sucrose and then starved for 16 h fed 12.0–16.0 times more often than flies kept on the other three feeding schedules (fig. 2a,b) (females: $F = 11.6$; d.f. = 3, 40; P < 0.0001; males: $F = 8.9$; d.f. = 3, 40; P < 0.0001). Females and males kept for 6 days on sucrose and then starved fed longer than flies kept on other feeding schedules (fig. 2c,d) (females: $F = 7.9$; d.f. = 3, 40; P = 0.0003; males: $F = 11.6$; d.f. = 3, 40; P < 0.0001). In females and males, there were no differences in durations of non-feeding events among treatments (fig. 2e,f).

When analysed with two-way ANOVA, feeding history had significant effects on numbers and durations of feeding events ($F = 19.5$; P < 0.0001; $F = 18.0$; P < 0.0001, d.f. = 3, 80; respectively), but not on durations of non-feeding events (P > 0.05). There was no sex effect on any of the variables and no feeding history × sex interactions (P > 0.05).

3.5 Responses to baits without and with spinosad

There were no significant differences in responses of 5- to 7-day-old flies to GF-120 without or with spinosad and no sex differences (table 5). However, at 20 h after exposure, there was a higher number of paralysed and dead flies in the GF-120 with than without spinosad treatment (with spinosad,

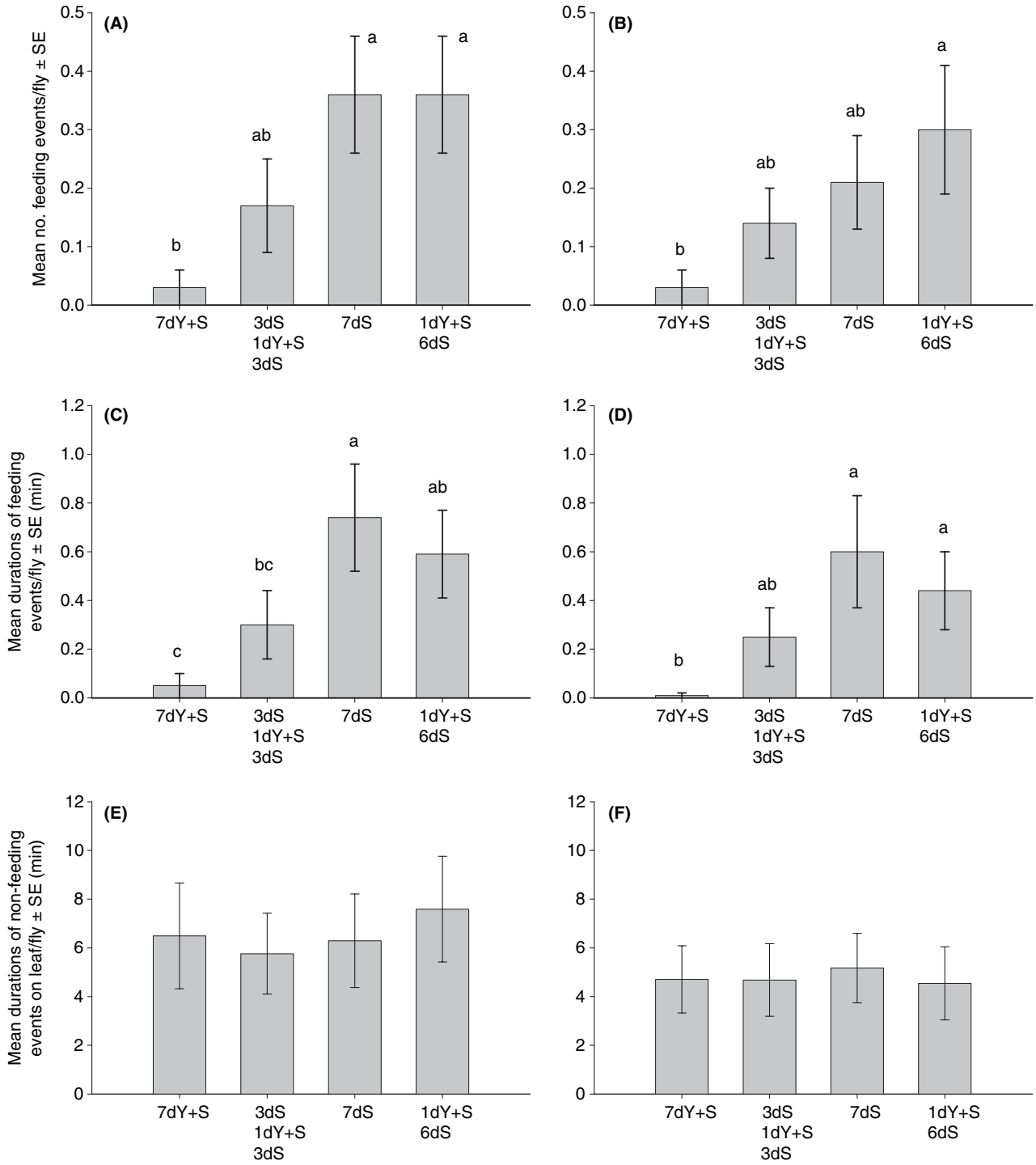

Fig. 1. *Responses of 8-day old* Rhagoletis indifferens *with different feeding history sequences and periods to Nulure: numbers of feeding events by (A) females and (B) males; durations of feeding events by (C) females and (D) males; durations of non-feeding events by (E) females and (F) males. d = day; Y + S = dry 20% yeast extract + 80% sucrose. Bars with same letter are not significantly different (P > 0.05)*

4.1 ± 0.3; without spinosad, 0.5 ± 0.2; $F = 78.8$; d.f. = 1, 18; $P < 0.0001$). There were also no significant differences in fly responses to Nulure without or with spinosad and no sex differences (table 5). However, at 20 h after exposure, there was a higher number of paralysed and dead flies in the Nulure with than without spinosad treatment (with spinosad, 3.7 ± 0.5; without spinosad, 0.8 ± 0.3; $F = 24.2$; d.f. = 1, 16; $P = 0.0002$).

3.6 Responses to fresh vs. aged GF-120 and Nulure

In 4- to 6-day-old flies kept on sucrose, there was no GF-120 age (0 and 24 h old) or sex effect on numbers of feeding events (fig. 3a), durations of feeding events (fig. 3b) and durations of non-feeding events (fig. 3c) ($P > 0.05$) (no age treatment × sex interaction, $P > 0.05$). Similarly, in 4- to 6-day-old flies kept on sucrose, there was no Nulure age (0 and 24 h old) or

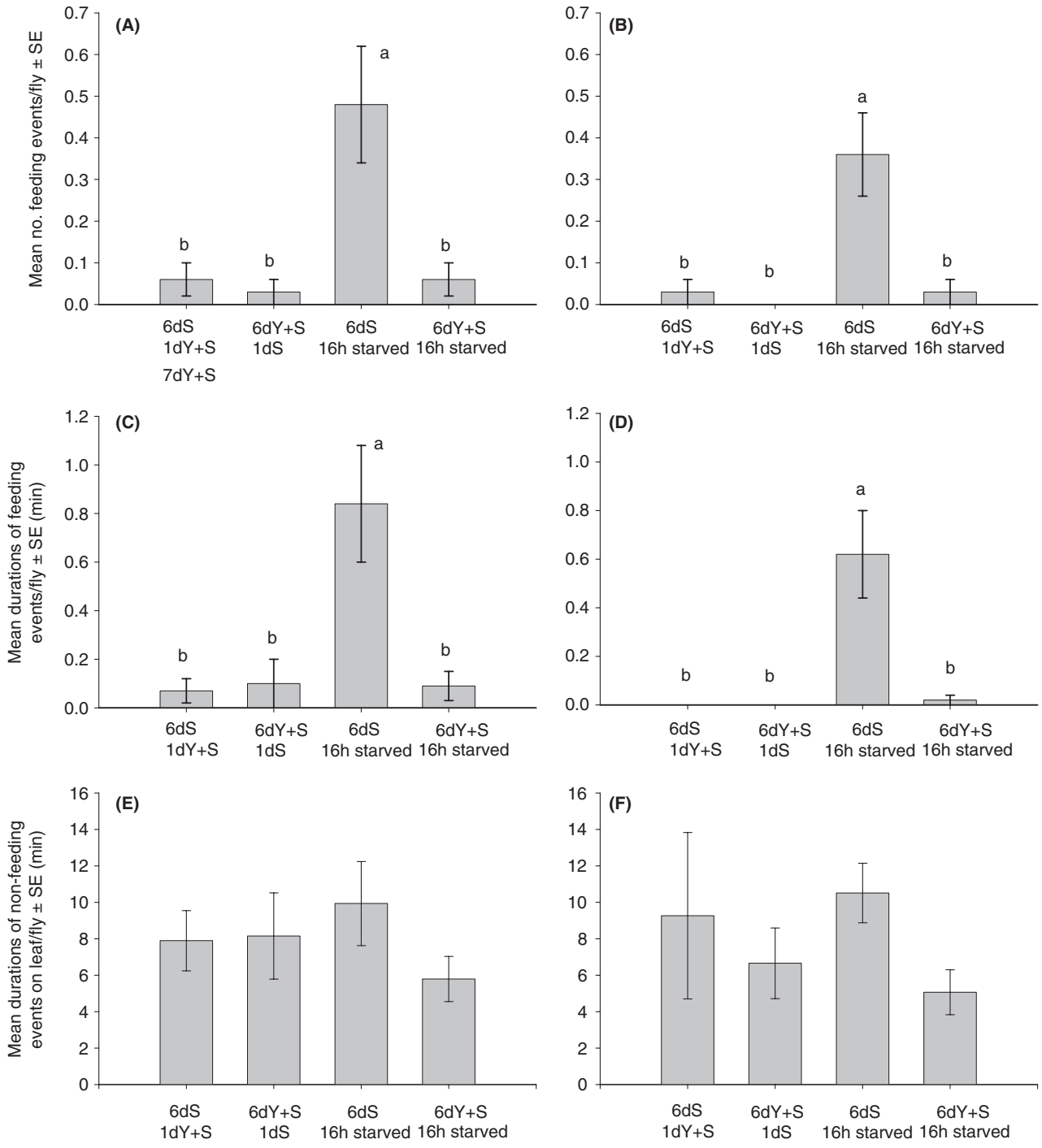

Fig. 2. *Eight-day-old* Rhagoletis indifferens *with different feeding histories and after starvation to Nulure: numbers of feeding by (A) females and (B) males; durations of feeding events by (C) females and (D) males; durations of non-feeding events by (E) females and (F) males. d = day; Y + S = dry 20% yeast extract + sucrose; S = 5% sucrose. Bars with same letter are not significantly different (P > 0.05)*

sex effect on numbers of feeding events (fig. 4a), durations of feeding events (fig. 4b) and durations of non-feeding events (P > 0.05) (fig. 4c) (no age treatment × sex interaction, P > 0.05).

There were no significant differences in durations of feeding events/fly on fresh GF-120 without and with spinosad: females, without spinosad, 0.07 ± 0.05 min/feeding event/fly; with spinosad, 0.17 ± 0.07 min; males, without spinosad, 0.25 ± 0.02 min; with spinosad, 0.02 ± 0.02 min (sex: $F = 0.04$; P = 0.8375; treatment: $F = 0.4$; P = 0.5127; sex × treatment: $F = 2.9$; P = 0.0969; d.f. = 1, 36). There were also no significant differences in durations of feeding events/fly on fresh Nulure without and with spinosad: females, without spinosad, 0.002 ± 0.002 min; with spinosad, 0.03 ± 0.03 min; males, without spinosad, 0.04 ± 0.03 min; with spinosad, 0.02 ± 0.01 min (sex: $F = 0.3$; P = 0.5805; treatment: $F = 0.0$; P = 0.9823; sex × treatment: $F = 1.0$; P = 0.3222; d.f. = 1, 36).

Table 5. *Feeding responses ± SE of 5- to 7-day-old* Rhagoletis indifferens *kept on 5% sucrose to 24-h-old GF-120 and Nulure without and with spinosad*

	No. feeding events/fly		Duration, feeding events/fly (min)		Duration, non-feeding events/fly (min)	
Treatment	Females	Males	Females	Males	Females	Males
Blank GF-120	1.10 ± 0.49	1.07 ± 0.62	0.23 ± 0.07	0.16 ± 0.11	4.51 ± 1.90	5.49 ± 1.84
GF-120 + spinosad	0.87 ± 0.32	0.53 ± 0.22	0.17 ± 0.06	0.12 ± 0.05	2.06 ± 0.63	3.06 ± 1.29
Two-way ANOVA*	*F*	P	*F*	P	*F*	P
Treatment	0.4	0.5575	0.7	0.4224	0.4	0.5160
Sex	0.4	0.5268	0.5	0.4827	2.6	0.1142
Treatment × sex	0.1	0.7885	0.01	0.9242	0.0	0.9937
Nulure	1.13 ± 0.49	0.57 ± 0.24	0.41 ± 0.23	0.22 ± 0.11	3.10 ± 1.63	0.70 ± 0.31
Nulure + spinosad	0.73 ± 0.25	0.83 ± 0.37	0.33 ± 0.17	0.18 ± 0.08	2.63 ± 1.08	3.04 ± 1.27
Two-way ANOVA*	*F*	P	*F*	P	*F*	P
Treatment	0.0	0.9463	0.1	0.7119	0.6	0.4299
Sex	0.4	0.5346	1.2	0.2770	0.7	0.4008
Treatment × sex	0.6	0.4605	0.01	0.9220	1.4	0.2406

*d.f. = 1, 36; ten replicates per treatment; three females and three males per replicate.

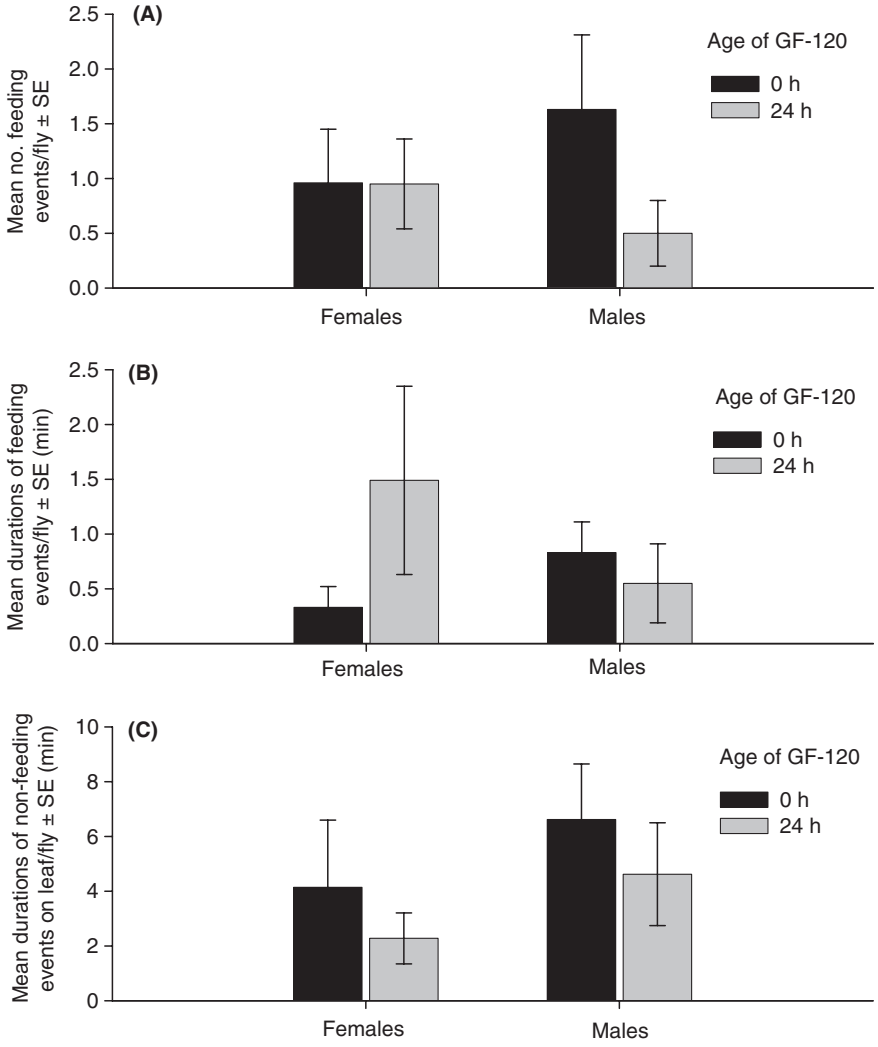

Fig. 3. *Responses of 4- to 6-day-old* Rhagoletis indifferens *kept on 5% sucrose to GF-120 aged 0 and 24 h. (a) Numbers of feeding events, (b) durations of feeding events, (c) durations of non-feeding events*

3.7 Feeding history and mortality of flies exposed to GF-120 and Nulure

Significantly higher numbers of flies kept on sucrose than flies kept on Y + S were paralysed or dead at 6–11 h after exposure to GF-120 with spinosad (table 6). Significantly higher numbers of flies kept on sucrose than flies kept on Y + S were paralysed or dead at 8–32 h after exposure to Nulure with spinosad (table 6).

4 Discussion

The first hypothesis that *R. indifferens* flies deprived of protein are more responsive to GF-120 than sucrose, and are more responsive than flies not deprived of protein was supported. Numbers of feeding events were consistently higher in protein-deprived flies. Because numbers of feeding events by 4- to 8-day-old

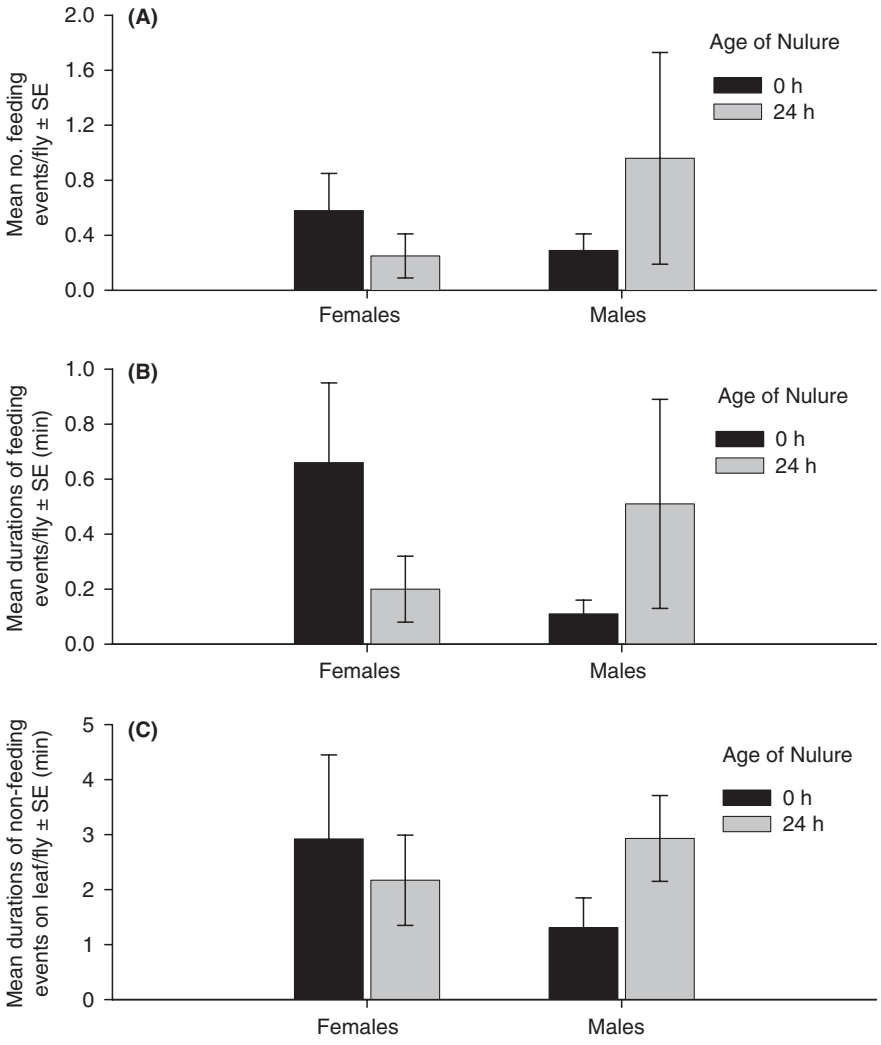

Fig. 4. *Responses of 4- to 6-day-old* Rhagoletis indifferens *kept on 5% sucrose to Nulure aged 0 and 24 h. (a) Numbers of feeding events, (b) durations of feeding events, (c) durations of non-feeding events*

Table 6. *Numbers of 5- to 8-day-old* Rhagoletis indifferens *of different feeding histories that were paralysed or dead ± SE at different hours after exposure to 24-h-old GF-120 (test 1) or Nulure (test 2) with spinosad on artificial leaves*

	No. flies paralysed or dead at hours after exposure						
Feeding history	2	4	6	8	11	24	32
Test 1: GF-120 + spinosad							
5% sucrose	0 ± 0	0.1 ± 0.1	0.7 ± 0.3	1.4 ± 0.4	2.5 ± 0.5	3.7 ± 0.6	4.5 ± 0.6
Y + S	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.2	2.6 ± 0.4	3.9 ± 0.2
One-way ANOVA, *F*	–	1.0	7.7	13.2	13.1	1.7	0.3
One-way ANOVA, P	–	0.3306	0.0124	0.0018	0.0020	0.2092	0.5790
Test 2: Nulure ± spinosad							
5% sucrose	0 ± 0	0.1 ± 0.1	0.4 ± 0.2	1.2 ± 0.4	2.3 ± 0.4	3.8 ± 0.4	4.7 ± 0.4
Y + S	0 ± 0	0 ± 0	0.1 ± 0.1	0.3 ± 0.2	0.5 ± 0.2	1.2 ± 0.4	1.9 ± 0.4
One-way ANOVA, *F*	–	1.0	2.4	4.3	17.1	19.6	19.2
One-way ANOVA, P	–	0.3306	0.1346	0.0520	0.0006	0.0003	0.0004

d.f. = 1, 36; Y + S: dry 20% yeast extract and 80% sucrose; ten replicates per treatment; three females and three males per replicate.

protein-deprived flies on GF-120 were greater than on sucrose (tables 1 and 2), it is possible there was some short-range attraction to the odour or sight of GF-120, although evidence for this was limited. Any attraction was low, as feeding events were highly variable even among individuals that were kept on sucrose alone. In some cases one of six flies within a replicate contributed to all feeding events on GF-120. However, this still represented 17% of flies that fed on drops over 1 h. Over a 24-h period, more than one fly would have been attracted to GF-120. This is supported by the numbers of flies that were paralysed or dead at 11 h after exposure to GF-120 with spinosad in experiment 7. An alternative to the attraction explanation is that protein-deprived flies incidentally encountered the GF-120 due to increased movement and then fed (also see next

paragraph). In an earlier study, 5- to 7-day-old *R. indifferens* kept on sucrose alone were not attracted to and did not feed on fresh GF-120 drops (Yee and Chapman 2005). The lack of responses may have been caused by the longer distances flies needed to move to find the GF-120 in that study, which was conducted inside a much larger cage. In the current study, the slightly longer feeding durations on GF-120 than on sucrose by 4- to 8-day-old flies (tables 1 and 2) suggest that the protein and sugar in GF-120 were more stimulatory to the protein-deprived flies than sucrose alone.

In 4- to 8- and 15- to 17-day-old flies, durations of non-feeding events by protein-deprived and protein-fed flies on sucrose- and GF-120-treated leaves can be interpreted in several ways with respect to attraction or feeding responses. Because sucrose releases no known olfactory cues detected by flies and durations of non-feeding events on sucrose- and GF-120-treated leaves were not different, 4- to 8-day-old flies may have found the sucrose drops through normal movement patterns typical of flies foraging. In addition to some weak attraction, it is also possible the protein-deprived flies found GF-120 through this mechanism. The effects of feeding on GF-120 on subsequent durations of stay on leaves need to be determined to fully support this hypothesis. In 15- to 17-day-old flies, overall durations of non-feeding events were greater on GF-120- than sucrose-treated leaves (table 1) possibly because there was some attraction to GF-120, even in protein-fed flies that were not hungry enough to feed on it. This hypothesis and the possible effects of fly age on durations of non-feeding events need further examination. There seemed to be no arrestment of activity caused by GF-120 in either sex (as defined by Kennedy 1978). In the field, however, GF-120 appeared to arrest *R. mendax*, which stayed longer within 5 cm of bait than water drops (Pelz et al. 2005).

Overall, the patterns in numbers and durations of feeding events on Nulure were similar to those on GF-120, but some differences were seen in non-feeding events of 4- to 8- and 15- to 17-day-old flies (tables 3 and 4). In 4- to 8-day-old flies, durations of non-feeding events on Nulure-treated leaves were greater than those on sucrose-treated leaves in both protein-deprived and protein-fed flies, suggesting that even protein-fed flies were attracted to Nulure, but not hungry enough to feed on it. In contrast, there were no differences in 15- to 17-day-old flies. Thus in at least these older flies, movement patterns appeared similar regardless of feeding history, bait or sex, suggesting Nulure was found and fed upon through chance encounters through normal movement. Whether there was a true age difference or whether the difference resulted from tests of the two age groups being conducted at different times is unclear. When durations of non-feeding events on leaves for both young and old flies were taken into account, there was no strong evidence that Nulure arrested flies.

The second hypothesis that females are more responsive to GF-120 and Nulure than males was not supported, even though in 4- to 8-day-old flies, numbers of feeding events were numerically greater in females (GF-120, $P = 0.0598$; Nulure, $P = 0.0996$). Feeding history sometimes affected the sexes differently, as indicated by interactions from the analyses. For example, females kept on sucrose fed more on GF-120 numerically than males kept on sucrose, although both sexes kept on Y + S did not feed on GF-120. Taken together, the results suggest sex response differences to protein baits may be present but difficult to detect, despite females needing more protein than males (at least in *R. pomonella*, Webster et al. 1979). In *R. pomonella*, females responded more to Nulure than males, but there were no detectable sex differences in relative responses to the control and Nulure (Prokopy et al. 1993), which is generally consistent with results in the current study.

The third hypothesis that flies deprived of protein for long periods respond more to Nulure than flies deprived for shorter periods was partially supported. The sequence of Y + S and sucrose exposure had clear effects on numbers and durations of feeding events. Flies kept 1 day on Y + S followed by 6 days on sucrose fed more than flies kept 3 days on sucrose, 1 day on Y + S, and 3 days on sucrose. Although not significantly different, this result hints at the possibility that flies of both sexes seek protein at least every 3 days and shows that protein is absolutely required for some function after 6 days. In *R. pomonella* females, protein feeding seems to be required every fourth day to maintain maximum fecundity (Hendrichs et al. 1990b). In the current study with *R. indifferens*, 1 day on Y + S after 6 days on sucrose shut down feeding responses, and missing 1 day of Y + S did not increase feeding, suggesting protein feeding does not need to occur every day. The similar durations of non-feeding events on leaves with Nulure suggest movement patterns by flies from all feeding histories were similar, although it is possible that after feeding, the durations of stay on leaves by protein-deprived flies were reduced.

The fourth hypothesis that flies starved after continuous sugar feeding respond more to Nulure than flies starved after continuous protein and sugar feeding was supported. Sixteen hours of starvation after sucrose feeding caused flies to feed more on Nulure than 16 h of starvation after Y + S feeding. Flies kept on Y + S starved for 16 h likely had higher energy reserves than flies kept on sucrose alone, resulting in an inhibition of their protein-feeding drive. The implication is that *R. indifferens* in nature with access to high amounts of protein may not feed on protein baits even after 16 h of no feeding. It is unknown how often feral *R. indifferens* are protein starved and for what duration. In *R. pomonella*, feeding responses of flies starved for 10 h best paralleled responses of flies in the field (Hu et al. 1999). Starvation in *R. pomonella* for 18–24 h did not reduce the ability of flies to find food (Malavasi and Prokopy 1992), suggesting nutrient reserves even after 1 day of starvation were sufficient for flies to find bait drops. Durations of non-feeding events on Nulure-treated leaves were not increased by starvation in females or males, suggesting movements of starved and non-starved flies were

similar or that after starved flies fed, their movements were reduced.

All previous experiments were conducted using bait without the toxin spinosad, but experiment 5 indicated that the presence of spinosad in GF-120 or Nulure had no significant effect on feeding responses of *R. indifferens*. This suggests flies were unable to detect the spinosad or that if they did detect it, its smell or taste was not deterrent. This is in agreement with findings in *R. mendax* (Barry and Polavarapu 2005).

Experiment 6 suggests *R. indifferens* kept on sucrose alone respond similarly to 0- and 24-h-old GF-120 or Nulure. This is in contrast to subtropical fruit flies, in which feeding responses to 0- or 1-h-old GF-120 were greater than to 2- or 5-hour-old GF-120 (Prokopy et al. 2003; Revis et al. 2004). This suggests that feeding responses of *Rhagoletis* species to fresh and aged protein baits differ from those of subtropical fruit flies. For *R. mendax*, there was no difference between numbers of flies that visited 0 to 2 and 3- to 4-day-old GF-120 on blueberry bushes (Pelz et al. 2005), suggesting the bait was not attractive fresh or aged. In addition, when fresh Solbait drops were placed 1 cm from *R. mendax* flies that had been starved for 16 h in the laboratory, only ~13% responded (Barry and Polavarapu 2004). Furthermore, the numbers of eastern cherry fruit fly, *Rhagoletis cingulata* Loew, observed on cherry trees treated with fresh GF-120 and with water, were not different, nor were the amounts of time spent by flies within 5 cm of fresh GF-120 or water droplets (Pelz-Stelinski et al. 2006). Perhaps the amounts of ammonia volatiles emitted when baits are 0 or 24 h old are below the threshold needed to elicit high responses from *R. indifferens*. Regardless of the possible differences of aging baits on responses by *Rhagoletis* species and subtropical fruit flies, this study is consistent with others (Hendrichs et al. 1990a; Prokopy et al. 1991, 1992, 1993, 1994; Robacker 1991; Vargas et al. 2002; Barry et al. 2003; Vargas and Prokopy 2006) in that it definitively showed protein deprivation increases the feeding responses of *R. indifferens* to protein baits.

Experiment 7 provided strong evidence that higher numbers of flies kept on sucrose alone fed on GF-120 or Nulure than flies kept on Y + S within the first 6 (GF-120) or 8 h (Nulure) after exposure. Flies kept on Y + S fed later on GF-120 than flies kept on sucrose, likely because their protein-feeding drive was delayed. In the Nulure test, few flies kept on Y + S apparently fed even at ≥24 h, perhaps because Nulure was less attractive or phagostimulatory than GF-120. Results suggest that feeding responses of protein-starved flies to GF-120 and Nulure are more immediate than those of protein-fed flies. Thus higher numbers of protein-deprived flies may be eliminated more quickly after bait applications, reducing chances these flies will survive long enough to find natural protein foods, develop eggs, and attack fruit.

For management of *R. indifferens*, the abundance of protein or high nitrogen foods such as bird droppings in cherry trees needs to be considered. As hypothesized by Prokopy et al. (1993), bait sprays should be more effective in trees with fewer bird droppings than in those with more. Reducing the abundance of birds that visit cherry trees may indirectly increase fly responses to baits. Highly attractive baits that entice even protein-fed flies to feed quickly or to prefer them over natural foods are needed to make bait sprays more effective. Other factors such as time of day, associated temperature changes and mating status also need to be studied to determine whether they interact with feeding history and how they may affect the feeding responses of *R. indifferens* to GF-120, Nulure and other protein baits.

In summary, this study increases our understanding of feeding history effects on the responses of *R. indifferens* to GF-120 and Nulure, and, as a consequence, of mechanisms of feeding responses. Overall, results show that complete protein deprivation, long periods of protein deprivation, and starvation after sucrose feeding increased fly feeding responses to GF-120 and Nulure. The general lack of differences in durations of non-feeding events on leaves with sucrose or GF-120 or Nulure in protein-deprived and protein-fed flies suggests that most protein-deprived flies found baits through chance encounters following normal movement. GF-120 or Nulure may work because of normal foraging, especially by protein-hungry flies, implying that effective management with baits requires high numbers of point sources that remain sufficiently toxic to kill flies for a prolonged period. Based on fly responses to GF-120 and Nulure, both should be equally effective in controlling flies, although direct comparisons of the two are needed to support this hypothesis.

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